PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 January 2001 (23.01.01)	
International application No. PCT/KR00/00535	**Applicant's or agent's file reference** P0020-SYH
International filing date (day/month/year) 25 May 2000 (25.05.00)	**Priority date** (day/month/year) 26 May 1999 (26.05.99)
Applicant HWANG, Seung-Yong et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 December 2000 (19.12.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia TEFY
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

LEE, Han Young

8th Fl., Seowon Bldg.,1675-1 Seocho-dong, Seocho-gu, Seoul 137-070, Republic of Korea

PCT

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)* 26 DECEMBER 2000 (26.12.2000)

Applicant's or agent's file reference

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/KR00/00535	25 MAY 2000 (25.05.2000)	26 MAY 1999 (26.05.1999)

Applicant

HWANG, Seung-Yong et al

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

19 DECEMBER 2000 (19.12.2000)

2. This date of receipt is :

[X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

[ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

[ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION**: That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*. Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/KR

Korean Industrial Property Office
Government Complex-Taejon, Dunsan-dong, So-ku, Taejon
Metropolitan City 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

COMMISSIONER

Telephone No. 82-42-481-5210

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

LEE, Han-Young
8th Floor, Seowon Building
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 23 January 2001 (23.01.01)	
Applicant's or agent's file reference P0020-SYH	**IMPORTANT INFORMATION**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/KR00/00535	25 May 2000 (25.05.00)	26 May 1999 (26.05.99)

Applicant
HWANG, Seung-Yong et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AE,AG,AL,AM,AT,AZ,BA,BB,BR,BY,CH,CR,CU,DK,DM,DZ,EE,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MW,MX,
   PT,SD,SG,SI,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
Olivia TEFY

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
LEE, Han-Young
8th Floor, Seowon Building
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

Date of mailing (day/month/year)	
07 December 2000 (07.12.00)	
Applicant's or agent's file reference P0020-SYH	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/KR00/00535	25 May 2000 (25.05.00)	26 May 1999 (26.05.99)

Applicant
HWANG, Seung-Yong et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   07 December 2000 (07.12.00) under No. WO 00/73505

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

LEE, Han-Young
8th Floor, Seowon Building
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

Date of mailing (day/month/year) 30 July 2000 (30.07.00)	
Applicant's or agent's file reference P0020-SYH	**IMPORTANT NOTIFICATION**
International application No. PCT/KR00/00535	International filing date (day/month/year) 25 May 2000 (25.05.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 26 May 1999 (26.05.99)
Applicant HWANG, Seung-Yong et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.
3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
26 May 1999 (26.05.99)	1999/19121	KR	28 June 2000 (28.06.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Max Germeil

Telephone No. (41-22) 338.83.38

PCT REQUEST

P0020-SYH

Original (for SUBMISSION) - printed on 25.05.2000 06:24:54 PM

0 0-1	For receiving Office use only International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4 0-4-1	Form - PCT/RO/101 PCT Request Prepared using	PCT-EASY Version 2.90 (updated 08.03.2000)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Korean Industrial Property Office (RO/KR)
0-7	Applicant's or agent's file reference	P0020-SYH
I	Title of invention	A METHOD FOR IDENTIFYING DNA MUTATION USING MICROWELL AND KIT THEREFOR
II II-1 II-2 II-4 II-5 II-6 II-7 II-8 II-9	Applicant This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence Telephone No. Facsimile No.	 applicant and inventor all designated States HWANG, Seung-Yong Sunkyung Apartment 103-603 Sa 2-dong, Ansan, Kyunggi-do 425-172 Republic of Korea KR KR 82-345-400-5516 82-345-419-1760
III-1 III-1-1 III-1-2 III-1-4 III-1-5 III-1-6 III-1-7	Applicant and/or inventor This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence	 applicant and inventor US only JUNG, Jin-Wook Samho Apartment 1-403 Bangbaebon-dong, Seocho-gu 137-069 Seoul Republic of Korea KR KR

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

LEE, Han-Young
8th Floor, Seowon Building
1675-1 Seocho-dong, Seocho-gu
Seoul 137-070
RÉPUBLIQUE DE CORÉE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
17 July 2000 (17.07.00)	
Applicant's or agent's file reference	**International application No.**
P0020-SYH	PCT/KR00/00535

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

HWANG, Seung-Yong (all designated States)
JUNG, Jin-Wook et al (for US)

International filing date	:	25 May 2000 (25.05.00)
Priority date(s) claimed	:	26 May 1999 (26.05.99)
Date of receipt of the record copy by the International Bureau	:	28 June 2000 (28.06.00)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EE,ES,
FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,
MG,MK,MN,MW,MX,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,
YU,ZA,ZW

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [X] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:

R. Chrem

Telephone No. (41-22) 338.83.38

003412428

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PCT REQUEST

P0020-SYH

Original (for SUBMISSION) - printed on 25.05.2000 06:24:54 PM

III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	KIM, Cheol-Min
III-2-5	Address:	Samik Apartment 211-811 148 Namchon 2-dong, Sooyoung-gu 613-012 Pusan Republic of Korea
III-2-6	State of nationality	KR
III-2-7	State of residence	KR
IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	LEE, Han-Young
IV-1-2	Address:	8th Fl., Seowon Bldg. 1675-1 Seocho-dong, Seocho-gu 137-070 Seoul Republic of Korea
IV-1-3	Telephone No.	82-2-596-7200
IV-1-4	Facsimile No.	82-2-596-7280
IV-1-5	e-mail	LeePat@hitel.net
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST

P0020-SYH

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V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	26 May 1999 (26.05.1999)	
VI-1-2	Number	1999-19121	
VI-1-3	Country	KR	
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1	
VII-1	**International Searching Authority Chosen**	Korean Industrial Property Office (KIPO) (ISA/KR)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description (excluding sequence listing part)	11	-
VIII-3	Claims	4	-
VIII-4	Abstract	1	p0020-pct(영문요약서).txt
VIII-5	Drawings	1	-
VIII-6	Sequence listing part of description	4	-
VIII-7	TOTAL	25	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		separate diskette
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	1	
VIII-19	**Language of filing of the international application**	Korean	

PCT REQUEST

P0020-SYH

Original (for SUBMISSION) - printed on 25.05.2000 06:24:54 PM

IX-1	Signature of applicant or agent	
IX-1-1	Name (LAST, First)	LEE, Han-Young

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/KR
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT

P0020-SYH

Original (for **SUBMISSION**) - printed on 25.05.2000 06:24:54 PM

PCT-EASY INFORMATION SHEET

(For applicant use only, DO NOT submit this sheet with the international application)

VALIDATION LOG

	Request
Green?	A translation of the international application into English will have to be prepared under the responsibility of the ISA selected.
Green?	Please note that the entire request (including the title of invention) must be in English
	Names
Yellow	Applicant 1.:City missing
	Fees
Yellow	Fee amount(s) should not equal zero.
	For receiving Office/International Bureau use only
Green?	Verify electronic data for consistency against printed form.

Before submitting the International Application, please carefully verify that:

-the information contained on printed Request form is correct;
-Box IX of the Request form has been signed;
-all elements of the international application as indicated in Box VIII of the Request form have been attached; and,
-the diskette containing the PCT-EASY zip file of the International Application has been enclosed and has been clearly labeled "PCT-EASY", with the applicant's or agent's file reference, and the first applicant's name.

ATTENTION

DO NOT modify any indications on the Request form printout. The attached PCT-EASY application has been locked. If an error or an omission is discovered at this time, you must copy the submitted application as a template and make the change or correction in a new application (using the submitted application as a template). You may create such a template by copying the submitted application from the "Stored Forms" folder to the "New PCT Forms" folder. Open the new (.0WO) file created in the "New PCT Forms" folder, correct the errors and proceed with the submission process again.

P0020-SYH

PCT (ANNEX - FEE CALCULATION SHEET)

Original (for **SUBMISSION**) - printed on 25.05.2000 06:24:54 PM

(This sheet is not part of and does not count as a sheet of the international application)

0 0-1	For receiving Office use only International Application No.	
0-2	Date stamp of the receiving Office	

0-4	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet				
0-4-1	Prepared using		PCT-EASY Version 2.90 (updated 08.03.2000)		
0-9	Applicant's or agent's file reference		P0020-SYH		
2	Applicant		HWANG, Seung-Yong, et al.		
12	Calculation of prescribed fees		fee amount/multiplier	total amounts (KRW)	
12-1	Transmittal fee	T	⇨	45,000	
12-2	Search fee	S	⇨	150,000	
12-3	International fee Basic fee (first 30 sheets)	b1	449,500		
12-4	Remaining sheets		0		
12-5	Additional amount	(X)	0		
12-6	Total additional amount	b2	0		
12-7	b1 + b2 =	B	449,500		
12-8	Designation fees Number of designations contained in international application		85		
12-9	Number of designation fees payable (maximum 8)		8		
12-10	Amount of designation fee	(X)	96,800		
12-11	Total designation fees	D	774,400		
12-12	PCT-EASY fee reduction	R	-138,300		
12-13	Total International fee (B+D-R)	I	⇨	1,085,600	
12-14	Fee for priority document Number of priority documents requested		1		
12-15	Fee per document	(X)	0		
12-16	Total priority document fee	P	⇨	0	
12-17	TOTAL FEES PAYABLE (T+S+I+P)		⇨	1,280,600	
12-19	Mode of payment		cash		

VALIDATION LOG AND REMARKS

13-2-1	Validation messages Request	Green? A translation of the international application into English will have to be prepared under the responsibility of the ISA selected.

PCT (ANNEX - FEE CALCULATION SHEET) P0020-SYH

Original (for **SUBMISSION**) - printed on 25.05.2000 06:24:54 PM

		Green? Please note that the entire request (including the title of invention) must be in English
13-2-3	Validation messages Names	Yellow Applicant 1.:City missing
13-2-7	Validation messages Fees	Yellow Fee amount(s) should not equal zero.
13-2-10	Validation messages For receiving Office/International Bureau use only	Green? Verify electronic data for consistency against printed form.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	SeeNotificationofTransmittalofInternationalPreliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR00/00535**	International filing date *(day/month/year)* 25 MAY 2000 (25.05.2000)	Priority date *(day/month/year)* 26 MAY 1999 (26.05.1999)

International Patent Classification (IPC) or national classification and IPC

IPC7 C12Q 1/68

Applicant

HWANG, Seung-Yong et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
19 DECEMBER 2000 (19.12.2000)	20 SEPTEMBER 2001 (20.09.2001)
Name and mailing address of the IPEA/KR Korean Intellectual Property Office Facsimile No.	Authorized officer AHN, Mi-Chung Telephone No. 042- 481-5593

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR00/00535

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[ ] the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statment) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language English which is

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[X] the language of publication of the international application(under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained inthe international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:
[ ] the description, pages _____
[ ] the claims, Nos. _____
[ ] the drawings, sheet _____

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION

International aplication No.
PCT/KR00/00535

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-18	YES
	Claims		NO
Inventive step (IS)	Claims	1-18	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-18	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

D1 : US 5,605,800

D2 : US 4,833,251

D3 : US 5,728,526

<Novelty & Inventive Step>

The invention defined by the claims is a method for identifying mutations in a tartget DNA, and a kit therefor. More specifically, the claimed invention comprises the steps of : 1) biotinylating the target DNA 2) affixing a probe comprising normal sequence of the target DNA to a microwell, 3) mixing the biotynylated target DNA with a probe attached to the microwell, 4) adding streptoavidine-linked degradation enzyme to that microwell, and 5) measuring biotin density bound to the microwell. The mutated target DNA can be distinguished with non-mutated DNA due to the different amount of absorbance detected.

D1 discloses a method for detecting the presence and characterizing a DNA or its sequence by employing of a chemically modified probe. Even though both of D1 and the present invention employ biotin-avidine reaction method for detecting the presence of specific DNA, the method in D1 is not designed to distinguish the specific mutation of target DNA. D2 relates to the compounds for tagging nucleic acid. This prior art demonstrates probes tagged with biotin used for the detection of nucleic acid hybridization. However, D2 does not teach the use of these probes for the identify the specific mutation of target DNA as shown in the claimed invention. D3 relates a method for analyzing a target nucleotide sequence. Even though the method in D3 permits the detection of single point mutation, the detailed technique employed in D3 is quite distinct from that in the claimed invention.

Since no individual citation or obvious combination of citations discloses the specific method for identifying mutations in a tartget DNA presented in the claimed invention, the novelty and the inventive step of the present invention can be acknowledged. Therefore, claims 1 to 18 satisfythe requirement of Article 33(2) and (3) of PCT.

<Industrial Applicability>

There is no reason to negative in the industrial applicability of the present invention.

Consequently, claims 1 to 18 appear to meet the requirement of Article 33(4) of PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
LEE, Han Young

8th Fl., Seowon Bldg.,1675-1 Seocho-dong, Seocho-gu, Seoul 137-070, Republic of Korea

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 28 SEPTEMBER 2001 (28.09.2001)

Applicant's or agent's file reference

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/months/year)*
PCT/KR00/00535	25 MAY 2000 (25.05.2000)	26 MAY 1999 (26.05.1999)

Applicant

HWANG, Seung-Yong et al

1. The applicant is hereby notified that International Preliminary Examining Authority transmits here with the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report(but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details in the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/KR
Korean Intellectual Property Office
Government Complex-Daejeon, Dunsan-dong, Seo-gu,
Daejeon Metropolitan City 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer

COMMISSIONER

Telephone No. 82-42-481-5210

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P0020-SYH		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/KR00/00535	25 MAY 2000 (25.05.2000)	26 MAY 1999 (26.05.1999)
Applicant		
HWANG, Seung-Yong et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
      ☐ contained in the international application in written form.
      ☐ filed together with the international application in computer readable form.
      ☐ furnished subsequently to this Authority in written form.
      ☐ furnished subsequently to this Authority in computer readable form.
      ☐ the statement that the subsequenlty furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
      ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawing** to be published with the abstract is Figure No. _____
   ☐ as suggested by the applicant.
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.
   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00535

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC C12Q 1/68, C07D 235/00, C12P 19/34, C07 21/04

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched
Korean Patents and Applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
IBM, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,605,800 (Institut Pasteur) 25 Feb 1997 see the whole document	1-18
Y	US 4,833,251 (Siska Diagnostics, Inc.) 23 May 1989 see abstract	1-18
Y	US 6,103,463 (The Public Health Research Institute of the City of New York, Inc.) 15 Aug 2000 see the whole document	1-18
Y	US 5,728,526 (Oncor, Inc.) 17 Mar 1998 see the whole document	1-18

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 SEPTEMBER 2000 (20.09.2000)	25 SEPTEMBER 2000 (25.09.2000)

Name and mailing address of the ISA/KR	Authorized officer
Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea	AHN, Mi-Chung
Facsimile No. 82-42-472-7140	Telephone No. 82-42-481-5593

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR00/00535

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5,605,800	25. 02. 97	US 5,955,262	21.09.99
		US 5,876,928	02.03.99
		US 4,581,333	08.04.86
		JP 3064119 B4	03.10.91
US 4,833,251	23. 05. 89	US 4,780,405	25.10.88
		US 5,210,203	11.05.93
		US 5,103,446	14.07.92
		JP 2014800 A2	23.01.87
		JP 2002164 A2	08.01.87
		EP 210021 A3	02.09.87
		EP 209996 A3	02.09.87
US 6,103,463	15. 08. 2000	EP 675966 A1	11.10.95
		WO 9317126 A1	02.09.93
		AU 3728093 A1	13.09.93
US 5,728,526	17. 03. 98	WO 9641001 A1	19.12.96
		AU 6150796 A1	30.12.96

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number
WO 00/73505 A1

(51) International Patent Classification[7]: C12Q 1/68

(21) International Application Number: PCT/KR00/00535

(22) International Filing Date: 25 May 2000 (25.05.2000)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
1999/19121 26 May 1999 (26.05.1999) KR

(71) Applicant and
(72) Inventor: **HWANG, Seung-Yong** [KR/KR]; Sunkyung Apartment 103-603, Sa 2-dong, Ansan, Kyunggi-do 425-172 (KR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **JUNG, Jin-Wook** [KR/KR]; Samho Apartment 1-403, Bangbaebon-dong, Seocho-gu, Seoul 137-069 (KR). **KIM, Cheol-Min** [KR/KR]; Samik Apartment 211-811, 148 Namchon 2-dong, Sooyoung-gu, Pusan 613-012 (KR).

(74) Agent: **LEE, Han-Young**; 8th Floor, Seowon Building, 1675-1 Seocho-dong, Seocho-gu, Seoul 137-070 (KR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/73505 A1

(54) **Title:** A METHOD FOR IDENTIFYING DNA MUTATION USING MICROWELL AND KIT THEREFOR

(57) **Abstract:** The present invention provides a method for identifying DNA mutation using microwell and kit therefor. The method for idenifying DNA mutation comprises the steps of: preparing biotin-bound nucleotide sequence amplified by PCR of a portion of DNA to be identified with biotin-bound primer; preparing a probe comprising normal sequence corresponding to the DNA to be identified; binding the probe with amine group of microwell; adding the biotin-bound nucleotide sequence to probe-bound microwell; adding a degradation enzyme to microwell to link the degradation enzyme with biotin of the probe; and, adding a substrate to be reacted with the degradation enzyme, and detecting the color or absorbance change caused by the degradation of the substrate. In accordance with the present invention, identification of mutated DNA can be realized in an economical, safe and simple manner, by employing PCR-amplified DNA and a probe of normal sequence corresponding to the DNA.

A METHOD FOR IDENTIFYING DNA MUTATION USING MICROWELLS AND KIT THEREFOR

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method for identifying DNA mutation using microwells and a kit therefor, more particularly, to an economical, simple and safe method for identifying single base mutations including substitution, insertion and deletion using microwells, and a kit therefor.

Backgound of the Invention

Techniques for determining the species of organisms and diagnosing diseases by identification of genes have been widely used in the art(see: J. Clin. Microbiol., 34:130-133, 1996). Diseases can be treated by using a variety of medications depending upon species and types of diseases. However, in such cases where the diseases are attributable to the infection with microorganisms which can be mutated, the use of conventional medication is limited.

Conventional techniques for detection of small mutations such as substitution, insertion and deletion in a DNA sequence includes dot blotting, RFLP(restriction fragment-length polymorphism) analysis, SSCP(single strand conformation polymorphism) analysis and DNA sequencing:

First, dot blotting technique employs the principle of Southern blotting, in which even a single base pair mismatch causes disruption of a DNA duplex above a certain temperature. This dot blotting can determine the presence of mutations through detection of signals created by a target sequence-represented oligonucleotide probe which has been prelabeled with a radioisotope, a fluorescent dye or

an enzyme connected to a chromogenic response when the oligonucleotide probe hybridizes nucleic acids immobilized on the membrane(see: British J. Dermatol., 131:72-77, 1994). Also, through the immobilization of an oligonucleotide probe on the membrane and preamplification of a target DNA, a mutation can be determined by the duplex fomation between the immobilized oligonucleotide and the amplified DNA.

Secondly, RFLP analysis employs the characteristic of restriction enzymes which cleave only specific sequences. In other words, a normal nucleotide sequence amplified by PCR can be cleaved with a restriction enzyme while the corresponding nucleotide sequence with mutations on the recognition site cannot be cleaved by the same enzyme. After treating normal and mutated DNA samples with a restriction enzyme, the resulting mixtures of DNA fragments are subject to electrophoresis side by side on the same gel, and the presence of mutation can be determined by comparing the number of DNA fragments from each sample(see: Mol. Cell Biol., 279-281, 1995).

Thirdly, SSCP analysis employs the characteristic of single-stranded DNA in which its conformational changes caused by even a single point mutation give rise to changes in migration of the fragments in a non-denaturing gel. When the pattern of the restriction cleavage of a normal DNA and that of a mutant DNA remain the same, it is sometimes possible to detect changes in sequence by their effects on the migration of short fragments of single-stranded DNA, in a technique called SSCP. By comparing the migration patterns of mutated and non-mutated DNA strand in the non-denaturing gel, the presence or absence of mutation can be determined(see: Mol. Cell Biol., 289, 1995).

In addition to several techniques mentioned above, mutations can be detected by direct DNA sequencing through gel electrophoresis or using instruments(see: Mol. Cell Biol., 245-248, 1995). The said techniques which typically involve the use of radioactive material, fluorescent dye or enzyme for detection of signals, have

drawbacks such as safty problems and requiring high cost in waste disposal in case of radioactive labeling, and if not radioactive labeling, cumbersome and time consuming manipulation due to the use of membranes or performing an acrylamide gel electrophoresis.

Meanwhile, a microwell plate which has been used for identifying a protein with an antibody or identifying a specific nucleotide sequence by hybidizing with DNA or oligonucleotide can be used for determining the presence or absence of a mutation by employing the method of identifying a specific nucleotide sequence. For instance, a sample DNA is affixed to the microwell, and then identified with an oligonucleotide probe corresponding to the normal DNA sequence to be identified: i.e., a sample DNA amplified by PCR technique is affixed to a microwell, hybridizes with a biotin-bound probe, followed by the assessment of hybridization using an enzyme(see: Mol. Cell Probes, 6(1):79-85, 1992). The prior art method is, however, proven to be less satisfactory in the senses that it takes much time to analyze a sample DNA since the sample DNA has to be affixed to the microwell after collecting from a patient; due to the length of DNA, a sample DNA amplified by PCR may have a secondary structure which cause inhibition of hybridization with a probe; long-length DNA molecule may prevent its binding to the well(see: Anal. Biochem., 138-142, 1991); and, since a sample DNA is affixed to a microwell after denaturation into single strands, renaturation of the single-stranded DNA takes place easily during the period of experiment, which lowers the binding ability of sample DNA to the probe.

Summary of the Invention

In accordance with the present invention, it has been discovered that the mutations of DNA sequence can be identified in an economical, simple and safe manner by using microwells and a degradation enzyme which can bind to

biotin.

A primary object of the present invention is, therefore, to provide a method for identifying mutations in a DNA sequence whose corresponding normal sequence has already been determined, by amplifying target(sample) DNA through PCR and using microwells.

The other object of the invention is to provide a kit therefor with convenience.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and the other objects and features of the present invention will become apparent from the following descriptions given in conjunction with the accompanying drawing, in which:

Figure 1 is a graph showing the results of identification of DNA mutations employing the method of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The method for identifying DNA mutation using microwells comprises the steps of: preparing amplified biotinylated DNA fragments of a portion of nucleotide sequence to be identified by PCR using a biotin-bound primer; preparing a probe comprising normal sequence corresponding to the DNA sequence to be identified; affixing the probe thus prepared to the amine group of microwell; adding biotinylated DNA fragments thus prepared to the probe-affixed microwell; adding a streptavidin-linked degradation enzyme to the microwell in order to bind the degradation enzyme to biotin moiety of the probe; and, adding a substrate to be reacted with the degradation enzyme and detecting the color or absorbance change caused by degradation of the substrate.

Also, a kit for identifying DNA mutation comprises a microwell whose inside has amine group, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC) solution and 1-methylimidazole solution, pH 7.0 for affixing a probe, 0.4M NaOH/0.25% Tween-20 solution for removing unaffixed probe, a solution containing dH_2O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml) for blocking microwell surface, a solution of 0.5xSSC/0.1% Tween-20 for removing unhybridized biotinylated sample DNA fragments, a streptavidin-linked degradation enzyme to be bound to biotin, 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl for binding of streptavidin to biotin, 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl/0.1% Tween-20 for removing unbound streptavidin, and a substrate for the streptavidin-linked degradation enzyme.

In describing the specification, the term of 'mutation' is employed to mean a sequence variation such as substitution in which one or more nucleotides are substituted with another nucleotide(s), deletion in which one or more of existing nucleotides are deleted, and insertion in which one or more of additional nucleotides are inserted.

Mutations caused by one or more nucleotide changes frequently occur in microorganisms. For example, Mycobacterium tuberculosis which has a nucleotide change at a specific position is known to be resistant to rifampicin(see: Lancet 341:647-650, 1993). Therefore, detection of the presence or absence of mutations, moreover, identification of changed nucleotide sequences may be very useful in treatment of patients.

In accordance with the present invention, the method for identifying DNA mutation can be applied to detect not only the presence of mutations but also identify the types of mutations such as nucleotide substitution, insertion and deletion with ease. The method involves the following steps of preparing amplified sample DNA, preparing a probe,

affixing the probe to a microwell and detection of mutation by binding of sample DNA to the probe.

Step 1: Amplification of sample DNA

A portion of DNA sequence to be identified for mutation is amplified by polymerase chain reaction(PCR) employing a biotin-bound primer to give amplified biotinylated DNA fragments of sample: Since the PCR is carried out by employing a biotin-bound primer, the amplified product by PCR is a mixture of biotinylated and non-biotinylated nucleotide sequences which are complementary to each other.

Step 2: Preparation of probes

DNA probes which comprise normal sequences corresponding to the DNA sequences to be identified are prepared: The probes thus prepared consist of more than 10 nucleotides and contains a phosphate moiety at 5'end which enables the probe to be affixed to the amine group of microwell.

Step 3: Affixture of probes

The probes thus prepared are affixed to amine group on the inner surface of microwell: Since nucleotide sequences of synthesized probe may be reannealed spontaneously, the single stranded probes can be maintained by heating the probes for 5 to 15 minutes, most preferably for 10 minutes, at 90 to 100℃, most preferably at 94℃, followed by immediate cooling in ice water. Ice cold solutions of 10mM 1-methylimidazole, pH 7.0 and 10 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), pH 7.0 which catalyze the covalent-binding reaction of phosphate moiety of oligonucleotide and amine group on microwell are added to the single-stranded DNA probe. The probe

containing mixture was added to the amine-derivatized microwell, and the microwells are sealed with tape to prevent evaporation of reaction mixture, followed by incubation for 5 to 9 hours, most preferably for 7 hours, at 40 to 60℃, most preferably at 50℃. In this way, the probes are covalently affixed on the inner surface of microwell. Probe-affixed microwells are washed with 0.4M NaOH/0.25% Tween-20, and then with distilled water at room temperature.

Step 4: Addition of sample to the microwell

Biotinylated PCR fragments obtained in the previous step are added to the probe-affixed microwells: First, a solution containing dH_2O, 20XSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml) is added to microwells, and incubated for 10 to 20 minutes, most preferably for 15 minutes, at 40 to 60℃, most preferably at 50℃, to prevent biotinylated sample from binding to free amine group on the microwell surface. In order to denature biotinylated double-stranded DNA fragments obtained in Step 1 to allow hybridization with the probe, they are heated for 5 to 15 minutes, most preferably for 10 minutes, at 90 to 98℃, most preferably at 94℃, and chilled immediately in ice water. And then, a solution containing dH_2O, 20XSSPE/0.0167% Triton X-100 and salmon sperm DNA (10mg/ml) is added to the single-stranded DNA sample. The mixture is introduced into the microwells, incubated for 5 to 15 hours, most preferably for 10 hours, at 50 to 70℃, most preferably at 60℃. After incubation, the residual mixture is removed, and microwells are washed with 0.5xSSC, 0.1% Tween-20. The sample with changed nucleotide sequence(s) would show low affinity for the probe affixed to microwell while the sample with normal sequence would show high affinity. Accordingly, biotin of the sample DNA fragments can be captured to the microwell, and the affinity difference between mutated and non-mutated sequences for

the probe can be measured in terms of the difference in density of microwell-bound biotin.

Step 5: Addition of a degradation enzyme

Streptavidin-linked degradation enzyme is added to the microwell in order to bind the degradation enzyme to biotin moiety of the probe-captured sample DNA fragment: To detect the difference in biotin density bound to the microwell of mutated and non-mutated DNA samples, degradation enzyme is bound to biotin. The degradation enzyme hydrolyzes a chromogenic substrate, and resulting changes in color intensity or absorbance can be measured. For example, streptavidin-alkaline phosphatase can be used; microwells are washed with a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20, and streptavidin-akaline phosphatase diluted with a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl, is added to the microwell, and incubated for 30 to 90 minutes, most preferably for 60 minutes, at 35 to 45℃, most preferably at 40℃. After removal of the residual reaction mixture, a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20 is added, incubated for 5 to 15 minutes, most preferably for 10 minutes, at 50 to 70℃, most preferably at 60℃, and then washed.

Step 6: Detection of enzyme reaction

The substrate for streptavidin-linked degradation enzyme was added to the microwell, and color or absorbance changes by enzyme reaction are detected: Synthetic peptides which show color or absorbance changes are desirable as substrates in degradation by the enzyme-substrate reaction. For example, in case of using streptavidin-alkaline phosphatase as a degradation enzyme, 100㎕ of pNPP(p-nitrophenyl phosphate) is added to the microwell, and

incubated for 60 to 120 minutes, most preferably for 90 minutes, at room temperature. After the reaction is stopped by addition of 1M NaOH, absorbance is measured at 405nm. Although the conventional spectrophotometer can be used to measure the absorbance, ELISA reader is a more desirable instrument to measure the absorbance of enzyme-substrate reaction mixture in microwells.

Also, the present invention provides a kit comprising the following components to implement the method described above: microwell whose inside has amine group, EDC solution and 1-methylimidazole solution, pH 7.0 for affixing a probe, 0.4M NaOH/0.25% Tween-20 solution for removing unaffixed probe, a solution containing dH_2O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml) for blocking free amine groups on microwell surface, a solution of 0.5xSSC/0.1% Tween-20 for removing unhybridized biotinylated sample DNA fragments, a streptavidin-linked degradation enzyme to be bound to biotin, 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl for binding of streptavidin to biotin, 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl/0.1% Tween-20 for removing unbound streptavidin, and a substrate for the streptavidin-linked degradation enzyme.

In accordance with the present invention, mutated and non-mutated DNA samples can be differentiated by measuring biotin density bound to the microwell. That is, a degradation enzyme is bound to biotin, and activity of the bound enzyme can be assayed by addition of a substrate reacting with the degradation enzyme. In this way, the presence of mutation in the sample DNA can be detected and, furthermore, the type of the mutation can be identified. Therefore, in any experiments to detect DNA mutations, the results can be obtained in a more economical, simpler and safer way than any other conventional method as long as the portion of DNA to be identified can be amplified and a probe comprising the normal sequence corresponding to the

DNA to be identified can be prepared.

The method for identifying DNA mutation using microwells of the present invention provides the following advantages over conventional methods:

1. Since the same results can be obtained by using smaller amount of oligonucleotides than conventional methods, the invented method is more economical. Similar results in terms of absorbance can be obtained with 100ng of probe/well by employing the method of the invention while prior art method requires 600ng/well(see: Mol. Cell. Probes, 12:407-416, 1998).

2. In the prior art, microwells pretreated for binding oligonucleotides later were provided. Instead, oligonucleotide-affixed microwells are provided in the present invention, which in turn reduces the costs for oligonucleotide synthesis and microwell preparation as well.

3. When mutation is detected by using a conventional microwell having amine group to which oligonucleotides are to be bound, high absorbance of negative control is a major problem. By using microwells of present invention, the absobance of negtive control can be reduced remarkably.

4. Conventional methods for detecting DNA mutation in which sample DNA is affixed to microwell have problems such as secondary structures caused by length of sample DNA, weak binding affinity and complementary binding between sample nucleotide strands. These problems are solved in the invention by preaffixing DNA probe to the microwell.

5. The prior art uses membranes or gels whose handling is very cumbersome, and requires many complicated work accompanying the difficulties in handling large number of samples, while the invented method guarantees simplicity by performing entire steps in one well.

6. The prior art has many variations such as different ways of collecting samples and conducting experiments, which is an obstacle to performing experiments in a reproducible manner. The kit of the invention not

only provides the consistency in experimental condition, but also can be stored for a long period of time, which enables detection of large number of samples at the same time. Especially, since experimental errors may be avoided by using instruments, experimental data or type of samples can be analyzed statistically.

7. Results can be identified visually. That is, color changes resulting from enzymatic reaction can be detected with naked eyes, therefore, due to the obviousness of the result, the reseacher can have confidence in the results.

The present invention is further illustrated in the following examples, which should not be taken to limit the scope of the invention.

Example 1: Identification of DNA mutation using microwells

The method for identifying DNA mutation of the invention involves procedures to detect the presence of mutation in patient's DNA by affixing a probe comprising the normal sequence corresponding to the DNA to be identified to the microwell, and then by assessing stability of the hybrid between a DNA sample and a complementary oligonucleotide probe immobilized on the microwell. As a representative example, the present inventors employed a direct sequenced region which is known to be uniquely found in genome of a Mycobacterium tuberculosis, BCG(Calmette-Guerin bacillus), and assessed the stability of duplexes of a sample DNA of a patient and various probes which include a probe of the normal nucleotide sequence of direct sequenced region, probes containing different types of mutations in the direct sequenced region, and a probe which is not related to the direct sequence as a negative control.

Example 1-1: The use of a probe comprising normal

nucleotide sequence

First, primer a comprising a biotin-bound nucleotide sequence of 5'-GGTTTTGGGTCTGACGAC-3' (SEQ ID: 1) and primer b of 5'-CCGACAGGGGACGGAAAC-3' (SEQ ID: 2) were synthesized by the conventional method. And then, PCR was performed in a PCR mixture containing DNA(comprising direct sequence of BCG) to a final concentration of 200 ng/㎕, 0.5㎕ of primer a(100pmol/㎕), 0.5㎕ of primer b(100pmol/㎕), 0.4㎕ of dNTP(25mM), 10xTaq buffer, 3㎕ of $MgCl_2$(25mM), 0.2㎕ of Taq(5unit/㎕, Promega, U.S.A.) and 40.4㎕ of dH_2O, with 30 cycles of denaturation(for 1min. at 94℃), annealing(for 1min. 30sec. at 55℃) and extension(for 1min. 30sec. at 72℃).

Subsequently, probe #1 comprising a normal nucleotide sequence of 5'-TTGACCTCGCCAGGAGAGAAGATCA-3' (SEQ ID: 3) and probe #2 comprising a normal nucleotide sequence of 5'-TCCGTACGCTCGAAACGCTTCCAAC-3') was synthesized. 10 pmol aliquots of each probe were heated for 10 minutes at 94℃, cooled down for 10 minutes in ice water, condensed by centrifugation. And then, a solution of EDC and 1-methylimidazole pH 7.0 was added to the each aliquot of the probe to a final concentration of 10mM. 100㎕ of each probe mixture thus prepared was introduced into microwells(Nunc, Denmark) on ice, incubated for 7 hours at 50℃. After removing the residual probe mixture, a solution containing 138㎕ of dH_2O, 20xSSPE/0.0167% Triton X-100, and 2㎕ of salmon sperm DNA(10mg/ml) was added to the microwells, and incubated for 20 minutes at 50℃ for blocking free amine group on the microwell surface.

After removing the blocking solution, a sample mixture containing 68㎕ of dH_2O, 30㎕ of 20xSSPE/0.0167% Triton X-100, 1㎕ of salmon sperm DNA(10mg/ml) and 1㎕ of PCR amplified sample DNA prepared above was added to each microwell, and incubated for about 10 hours at 60℃. After removing the mixture, each microwell was washed 3 times with 200㎕ of 0.5x SSC, 0.1% Tween-20, and the same

solution was added to incubate for 15 minutes at 60℃. After washing 3 times with the same solution, 100 ㎕ of streptavidin-alkaline phosphatase prepared by 3000-fold dilution with a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl was added to the microwell, and incubated for 1 hour at 40℃.

Subsequently, the microwells were washed 3 times with a buffer solution of 100mM Tris-HCl (pH 7.5) containing 150mM NaCl/0.1% Tween-20, and subjected to 3 times of incubation for 5 minutes at 60℃ and washing with the same solution. 100 ㎕ of pNPP(N7653, Sigma, U.S.A) was added to each well, incubated for 90 minutes, and then the absorbance was measured at 405nm(see: Figure 1).

Example 1-2: The use of a probe with a substituted nucleotide

To identify a substitution mutation, probe #11 of 5'-TTGACCTCGCCAGAAGAGAAGATCA-3'(SEQ ID: 5) was synthesized by substituting the 14th nucleotide of probe #1, G with A, and probe #21 of 5'-TCCGTACGCTCGAGACGCTTCCAAC-3'(SEQ ID: 6) was synthesized by substituting the 14th nucleotide of probe #2, A with G. Then, identification of DNA mutation was performed in an analogous manner as in Example 1-1, except for employing the use of probes #11 and #21 instead of probes #1 and #2(see: Figure 1).

Example 1-3: The use of a probe with an inserted nucleotide

To identify an insertion mutation, probe #12 of 5'-TTGACCTCGCCAGTGAGAGAAGATCA-3'(SEQ ID: 7) was synthesized by inserting T between 13th and 14th nucleotide of probe #1. Then, identification of DNA mutation was performed in an analogous manner as in Example 1-1, except for employing the use of probe #12 instead of probes #1 and #2(see: Figure 1).

Example 1-4: The use of a probe with a deleted nucleotide

To identify a deletion mutation, probe #13 of 5'-TTGACCTCGCCAGAGAGAAGATCA-3'(SEQ ID: 8) was synthesized by deleting 14th nucleotide, G of probe #1. Then, identification of DNA mutation was performed in an analogous manner as in Example 1-1, except for employing the use of probe #13 instead of probes #1 and #2(see: Figure 1).

Example 1-5: The use of a negative control probe

For a negative control, probe #C of 5'-GGAGCTTTCCGGCTTCTATCAGGTA-3'(SEQ ID: 9) comprising a nucleotide sequence which is found uniquely in a Mycobacterium tuberculosis, H37Rv, was synthesized. Then, identification of DNA mutation was performed in an analogous manner as in Example 1-1, except for employing the use of probe #C(see: Figure 1).

Figure 1 shows a graph, based on a direct sequenced region which is known to be uniquely found in the genome of Mycobacterium tuberculosis, BCG, demonstrating the identification of DNA mutation by assessing the stability of the duplex of a sample DNA from a patient and various probes which include a probe comprising a normal nucleotide sequence of direct sequenced region, probes containing different types of mutations in the direct sequenced region, and a probe which is not related to the direct sequence as a negative control. In Figure 1, No DNA represents a control without probe; No.1, a negative control, probe #C; No.2, probe #1 of normal sequence; No.3, probe #11 of substitution mutant; No.4, probe #13 of deletion mutant; No.5, probe #12 of insertion mutant; No.6, probe #2 of normal sequence; and, No.7, probe #21 of substitution mutant, respectively.

As shown in Figure 1, since the OD value from the

hybridization of the sample DNA with the probe of normal sequence showed the highest value, the sample DNA from a patient turned out to comprise the normal sequence. On the other hand, although the hybridization of a sample DNA with probes of mutated sequences made differences in OD values for each mutated probe more or less, the values were significantly lower than that for a probe of normal sequence, thus assuring that the presence of mutation can be clearly identified by the method of the invention.

The application of the invention make it possible to identify the position and the type of mutation by employing probes comprising each mutated sequence with one or more nucleotide changes.

As clearly illustrated and demonstrated above, the present invention can be applied to identify not only the presence of one or more mutations but also the types of mutations such as substitution, deletion and insertion in a more economical, simpler and safer way than any other conventional method.

WHAT IS CLAIMED IS:

1. A method for identifying DNA mutation using microwells which comprises the steps of:

(i) preparing amplified biotinylated DNA fragments of a portion of nucleotide sequence to be identified by PCR using a biotin-bound primer;

(ii) preparing a probe comprising normal sequence corresponding to the DNA sequence to be identified;

(iii) affixing the probe prepared in Step(ii) to the amine group of microwell;

(iv) adding biotinylated DNA fragments prepared in Step(i) to the probe-affixed microwell;

(v) adding a streptavidin-linked degradation enzyme to the microwell in order to bind the degradation enzyme to biotin moiety of the probe-captured sample DNA fragment; and,

(vi) adding a substrate to be reacted with the degradation enzyme and detecting the color or absorbance change caused by degradation of the substrate.

2. The method for identifying DNA mutation of claim 1, wherein the probe is a nucleotide sequence consisting of more than 10 nucleotides and containing a phosphate moiety at 5' end.

3. The method for identifying DNA mutation of claim 1, wherein Step(iii) comprises adding single-stranded probe to the microwell, adding catalysts in order to bind the phosphate moiety of the single-stranded probe to the amine group of microwell, and washing the microwell.

4. The method for identifying DNA mutation of claim 3, wherein the catalysts are ice-cold solutions of 10mM 1-methylimidazole, pH 7.0 and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), pH 7.0.

5. The method for identifying DNA mutation of claim 3, wherein the washing is performed by employing 0.4M NaOH/0.25% Tween-20 solution.

6. The method for identifying DNA mutation of claim 1, wherein Step(iv) comprises binding single-stranded DNA fragments obtained in Step(i) to the probe of microwell, removing the residual DNA fragments, and washing the microwell.

7. The method for identifying DNA mutation of claim 6, further comprising pretreatment of the microwell with a solution containing dH_2O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml) at 50℃ for 20 minutes before adding the single-stranded DNA fragments obtained in Step(i) to the microwell.

8. The method for identifying DNA mutation of claim 6, wherein binding the DNA fragments obtained in Step(i) to the probe occurs in a solution containing dH_2O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml).

9. The method for identifying DNA mutation of claim 6, wherein the washing is performed by employing 0.5xSSC/0.1% Tween-20 solution.

10. The method for identifying DNA mutation of claim 1, wherein Step(v) comprises the first washing of the microwell, introducing streptavidin-linked degradation enzyme to the microwell for binding of the enzyme with biotin, removing the residual reaction mixture, and the second washing of the microwell.

11. The method for identifying DNA mutation of claim 10, wherein the first washing is performed by employing a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20.

12. The method for identifying DNA mutation of claim 10, wherein the streptavidin-linked degradation enzyme is streptavidin-alkaline phosphatase dissolved in a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl.

13. The method for identifying DNA mutation of claim 10, wherein the second washing comprises treatment of the microwell with a buffer solution of 100mM Tris-HCl(pH 7.5) containing 150mM NaCl/0.1% Tween-20 at 60℃ for 10 minutes.

14. The method for identifying DNA mutation of claim 1, wherein the substrate to be reacted with streptavidin-linked degradation enzyme is a synthetic peptide showing color or absorbance change during the degradation.

15. The method for identifying DNA mutation of claim 14, wherein the substrate is pNPP(p-nitrophenyl phosphate) provided that the streptavidin-linked degradation enzyme of streptavidin-alkaline phosphatase is employed.

16. The method for identifying DNA mutation of claim 1, wherein the color change is detected with naked eyes.

17. The method for identifying DNA mutation of claim 1, wherein the absorbance is measured by employing an ELISA reader.

18. A kit for identifying DNA mutation which comprises:

(i) a microwell whose inside has amine group;

(ii) 10mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), pH 7.0 and 10mM 1-methylimidazole, pH 7.0;

(iii) 0.4M NaOH/0.25% Tween-20 solution;

(iv) a solution containing dH_2O, 20xSSPE/0.0167% Triton X-100 and salmon sperm DNA(10mg/ml);

(v) 0.5xSSC/0.1% Tween-20 solution;

(vi) streptavidin-alkaline phosphatase;

(vii) 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl;

(viii) 100mM Tris-HCl(pH 7.5) solution containing 150mM NaCl/0.1% Tween-20; and,

(ix) pNPP(p-nitrophenyl phosphate).

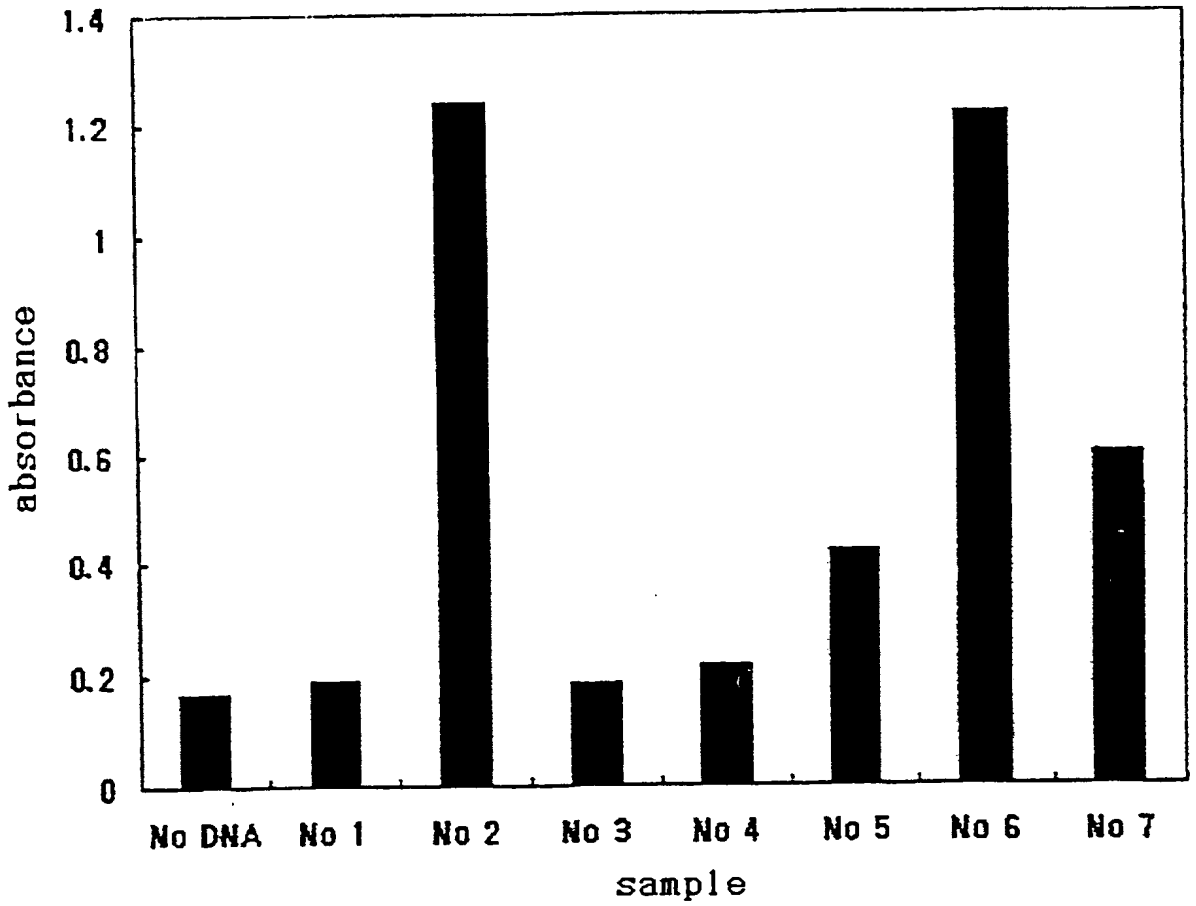
1.4
1.2
1
0.8
0.6
0.4
0.2
0
absorbance
No DNA
No 1
No 2
No 3
No 4
No 5
No 6
No 7
sample

Fig. 1